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GRANT NUMBER DAMD17-96-1-6056

TITLE: Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms

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REPORT DATE: July 1998

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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DTIC QUALITY INSPECTED 4

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 1998	3. REPORT TYPE AND DATES COVERED Annual (15 Jun 97 - 14 Jun 98)	
4. TITLE AND SUBTITLE Fatigue in Persian Gulf Syndrome-Physiologic Mechanisms			5. FUNDING NUMBERS DAMD17-96-1-6056	
6. AUTHOR(S) Ronald G. Haller, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Southwestern Medical Center at Dallas Dallas, Texas 75235-9105			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES <div style="text-align: right; font-size: 2em; font-weight: bold;">19990610138</div>				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) We hypothesize that the common complaint of abnormal fatigue and exercise intolerance in these patients is attributable to impaired energy production via oxidative phosphorylation. Under this general hypothesis, we will address three specific questions: 1) Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals? 2) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production? 3) Is the metabolic and physiologic response to aerobic physical conditioning impaired in these patients. In order to address these questions, we will employ forearm and cycle exercise to determine maximal work and oxidative capacity and to compare fatigue and metabolic responses to similar relative workloads among patients and age and weight matched sedentary control subjects; and we will compare muscle metabolic and physiologic responses to aerobic training in patients and matched control subjects. We will monitor oxidative metabolism by employing 31-phosphorus magnetic resonance spectroscopy; and by utilizing near infrared spectroscopy. In a cohort of patients and control subjects we will evaluate the hypothesis that oxidative limitations detected with non-invasive testing is attributable to impaired function of the mitochondrial metabolism as assessed biochemical in biopsied muscle.				
14. SUBJECT TERMS Gulf War Illness			15. NUMBER OF PAGES 13	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

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Ronald J. Haller 7/28/98
PI - Signature Date

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Introduction

A variety of illnesses have been linked to military service in the Persian Gulf Conflict, but no consistent medical syndrome has been recognized and no specific etiology is known. Muscle symptoms, in particular abnormal fatigability and myalgias, are common in affected individuals, but the etiology of these muscle symptoms is unknown. We are investigating the general hypothesis that a fundamental physiologic mechanism of muscle fatigue in Gulf War veterans is an impairment of muscle oxidative metabolism. Under this general hypothesis, we are addressing four specific questions:

- 1) Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production?
- 2) Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals?
- 3) Is the normal increase in muscle oxygen utilization and the capacity for oxygen transport in response to regular, aerobic physical conditioning impaired in these patients?
- 4) Is there a specific pattern of impaired activities of mitochondrial enzymes to account for impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training?

Our study will enroll 25 Gulf War veterans with prominent symptoms of fatigability and myalgia and matched control subjects employing resources of a center devoted to the study of human muscle metabolic disorders and to investigation of the physiologic basis of chronic fatigue.¹⁻³ The study employs protocols and non-invasive monitors of oxygen transport and utilization as well as detailed muscle biochemistry employed in our laboratory to identify specific causes of exercise intolerance in patients referred to our center. These include measurement of systemic oxygen transport (cardiac output) at rest and in exercise by means of acetylene rebreathing;⁴ monitoring of muscle metabolism by ³¹ phosphorus magnetic resonance spectroscopy;⁵⁻⁷ and monitoring of muscle oxygenation by means of near infrared spectroscopy.^{8, 9}

Body

Experimental methods, procedures:

Subject identification, recruitment: . We have proposed to identify and recruit 25 affected veterans and 25 control subjects.

Experimental procedures: The fundamental approach to evaluating muscle oxidative metabolism in Gulf War veterans involves cycle and forearm exercise testing during which physiologic and metabolic changes related to muscle oxidative capacity are monitored:

a) *Cycle exercise.* Subjects undergo resting and exercise evaluation of oxidative metabolism using cycle ergometry. Studies are designed to assess peak capacity for oxygen utilization and oxygen transport (cardiac output) as well as monitoring changes in blood levels of metabolites that reflect levels of anaerobic glycogenolysis (blood lactate and lactate/pyruvate ratios) as well as heart rate and blood pressure responses to graded exercise.

b) *Aerobic forearm exercise.* Subjects also undergo aerobic forearm exercise monitoring the pattern of contractile fatigue and changes in venous effluent metabolites that reflect the rate of glycogenolysis and adenine nucleotide breakdown via adenylate deaminase.

c) *Near infrared spectroscopy (NIRS)* . Direct evaluation of oxygen extraction over working muscle is evaluated utilizing NIRS performed during repetitive hand gripping exercise sampling oxygenation of the flexor digitorum profundus. Light in the NIR range (700-1000 nm) passes readily through biological tissues including skin and bone. NIR light is diffusely scattered by tissues and photons are absorbed primarily by the iron-porphyrin complexes of oxy- and deoxyhemoglobin and -myoglobin and by oxidized copper atoms of cytochrome aa3. NIR is able to detect *qualitative* changes in the reduction-oxidation state of the copper complex of cytochrome aa3 (in cytochrome c oxidase) and oxygenation of tissue hemoglobin (Hgb) and myoglobin (Mgb). Thus, NIR spectroscopy provides a unique opportunity to evaluate noninvasively local muscle O₂ extraction and the state of mitochondrial redox in muscle relative to oxygen extraction from circulating blood and myoglobin. This technique permits detection of muscle oxidative defects attributable to inborn errors of metabolism.^{9, 10}

d) *³¹P Phosphorus magnetic resonance spectroscopy (³¹P MRS).* ³¹P MRS permits measurement of *intracellular* metabolites of relevance to muscle energy metabolism. 5 major phosphorus peaks are found in resting muscle: orthophosphate (Pi), phosphocreatine (PCr), and 3 peaks corresponding to the α , β , and γ phosphates of ATP. Peak height and area correlate with the relative concentrations of the respective metabolite.^{11, 12} The β peak typically is used to estimate concentrations of ATP and by convention is assumed to represent 5.5 mM per kgm wet weight of muscle.^{13, 14} Phosphorus MRS has identified a number of abnormalities in patients with respiratory chain defects. At rest muscle PCr levels are often low

and Pi may be elevated.¹⁵⁻¹⁸ This result has been interpreted to indicate that oxidative phosphorylation is deficient even at rest. With exercise there is typically an exaggerated fall in PCr and rise in Pi relative to work performed consistent with impaired oxidative phosphorylation. After exercise, recovery of PCr typically is greatly delayed, consistent with the oxidative deficit.

e) *Muscle biopsy - histochemical and biochemical evaluation.* We have proposed to obtain skeletal muscle for histological and biochemical analysis utilizing the needle biopsy technique from symptomatic and asymptomatic veterans in order to determine whether specific enzyme deficiencies underlie limitations of exercise and oxidative capacity. In addition the muscle enzymatic response to training will be correlated with changes in exercise capacity after aerobic conditioning in 10 symptomatic and 10 asymptomatic veterans.

f) *Aerobic training* - we have proposed to enroll 10 patients and 10 control subjects in a 10 week period of training to assess physiologic and metabolic adaptation and to test the hypothesis that the subjects with symptoms of fatigability show altered capacity to adapt to conditioning exercise.

Results and Discussion

Subject identification, recruitment: . We have employed a factor analysis of symptoms in collaboration with Dr. Robert Haley, Chief of Epidemiology, University of Texas Southwestern Medical Center.¹⁹ In addition, in the attempt to correlate neuromuscular symptoms with possible toxin exposure during service in the Persian Gulf conflict, we have administered a survey of toxin exposure developed and employed by Dr. Haley in recently published studies of the epidemiology of the Persian Gulf syndrome.²⁰ These instruments were initially administered to 107 veterans, including both well and symptomatic individuals, who live in the North Texas area and are followed at the Dallas VA Medical Center. 18 symptomatic and asymptomatic candidates for the study were identified. From this number we have been able to recruit and study 24 veterans - 13 with prominent symptoms of muscle fatigue or myalgias and 11 asymptomatic veterans (controls). These subjects are well matched with respect to age, weight and height (Table 1).

Table 1: characteristics of symptomatic (patients) and asymptomatic (controls) GW veterans (Mean±SEM).

variables	patients (n = 13)	controls (n = 11)	p value (unpaired t test)
age	41±3	39±3	0.5775
height (cm)	173.1±2	176.3	0.2993
weight (kg)	84.7±5.5	86.5±2.6	0.7731

In the past year, survey instruments have been administered to an additional 200 veterans followed at the Dallas VA Medical Center from whom the remainder of veterans with neuromuscular symptoms and asymptomatic control subjects will be recruited to complete this study.

Advantages of this approach to subject identification includes increased likelihood of identifying a consistent pattern of neuromuscular symptoms and exercise pathophysiology; establishing a mechanism for identifying possible specific links between exposure to specific risk factors and symptoms; and, by identifying local veterans, we improve subject accessibility which is of particular importance for the training phase of the study. The disadvantage in implementing this changed design of our protocol has been a delay in enrollment of subjects in exercise studies which has necessitated a request for a no cost extension of this grant.

Data addressing specific hypotheses:

1. Is there exaggerated metabolic muscle fatigue in exercise consistent with impaired energy production?

Abnormal fatigability has been the dominant neuromuscular symptom in the veterans that we have studied. We have attempted to provide objective evaluation of the question of abnormal fatigability by evaluating work capacity and fatigability during forearm and cycle exercise. Data compiled to date indicates similar work capacity and fatigue rates with hand grip exercise but reduced work capacity in cycle exercise in patients compared to control subjects:

a) Values for peak grip force at rest and throughout exercise and for peak post exercise levels of lactate and ammonia were lower for patients (Table 2), but none of these differences reached statistical significance. Furthermore, expressed as a percentage of initial force, values at 1, 30, and 60 seconds of aerobic handgrip were virtually identical in patients and control subjects indicating similar rates of fatigue with this exercise.

Table 2: Aerobic forearm exercise (mean±SEM)

variables	patients (n = 13)	controls (n = 11)	p value (unpaired t test)
initial force (kg)	40.7±3.3	44.6±3.7	0.4366
force at 1 sec	37.3±2.6	43.0±3.8	0.2226
% of initial at 1 sec	93±.03	96±.03	0.5561
force at 30 sec	31.7±2.5	35.9±2.9	0.2682
% of initial at 30 sec	81±.04	81±02	0.9604
force at 60 sec	28.1±2.2	31.1±2.3	0.3593
% of initial at 60 sec	70±.03	71±.03	0.8484
peak lactate p exercise (mM)	4.06±0.30	4.12±0.33	0.8863
peak ammonia p exercise (µM)	41.9±7.7	60.8±12.1	0.1937

b) In contrast to the results for hand grip exercise, we have identified a significantly lower work capacity in cycle exercise in patients compared to control subjects (table 3). Peak work in patients was 124±7 watts whereas peak work capacity in control subjects was 163±15 watts ($p<.02$). This lower work capacity was not explained by a lower effort since peak exercise heart rate was similar in both subject groups (table 3).

Table 3: Oxygen uptake, extraction, and transport during peak cycle exercise (mean±SEM).

variables	patients (n = 13)	controls (n = 11)	p value (unpaired t test)
peak heart rate (bpm)	167±5	169±3	0.7975
peak work load (watts)	124±7	163±15	0.0197
peak VO ₂ (L/min)	2.03±0.12	2.39±0.15	0.0687
peak cardiac output (L/min)	16.3±0.5	17.4±0.89	0.2491
peak systemic arteriovenous diff	12.49±0.66	13.60±0.59	0.2362
Q/ VO ₂	6.3±0.6	5.7±0.3	0.3275
Diastolic BP	86±3	84±2	0.8048
Systolic BP	132±5	133±3	0.5172
Mean arterial pressure	101±3	100±2	0.8048
RER	1.134±0.024	1.097±0.019	0.2468
peak lactate (mM)	7.05±0.607	7.98±0.69	0.3259
peak pyruvate (mM)	0.290±0.013	0.324±0.012	0.0695
peak L/P	26.85±1.36	27.72±1.18	0.6348
peak K ⁺ (mM)	5.4±0.2	5.6±0.2	0.3985

2. Is there an abnormality of muscle oxygen utilization or oxygen transport to muscle during exercise in affected individuals?

We have addressed this question by employing cycle ergometry and measuring oxygen utilization and cardiac output (systemic O₂ transport) during peak exercise and by calculating peak systemic arteriovenous O₂ difference. Mean values for oxygen utilization, cardiac output, and systemic a-v O₂ difference were all lower in patients compared to controls, but none of these differences reach statistical significance (Table 3). Similarly there were no statistically significant differences in peak venous lactate, pyruvate, and in the lactate/pyruvate (L/P) ratio (Table 3). The fuel mix of oxidative metabolism as reflected in the RER was also similar between patient and control subjects (Table 3). We also have evaluated the integrity of muscle oxygen extraction relative to oxygen delivery during forearm exercise utilizing near infrared spectroscopy. No qualitative differences in NIR spectroscopy have been identified in the 24 subjects studied to date.

3. Is the increase in muscle oxygen utilization and in the capacity for oxygen transport in response to regular, aerobic physical conditioning impaired in these patients?

We have enrolled 7 veterans (5 patients, 2 controls), 4 of whom (2 patients, 2 controls) have completed 10 weeks of training. All of the subjects who have completed the aerobic conditioning phase of this study have demonstrated an increased oxidative capacity attributable to increased cardiac output or increased peak systemic a-v O₂ difference (Table 4).

Table 4: Peak cycle exercise pre and post training.

subject	pt/cont	peak work (watts)	peak VO ₂ (L/min)	peak Q (L/min)	peak a-v O ₂ diff (ml/dl)
RM pre	pt	140	2.27	15.87	14.3
RM post		170	2.44	17.63	13.8
FS pre	pt	115	2.17	16.43	13.2
FS post		140	2.33	17.61	13.2
PR pre	cont	200	3.26	20.34	16.0
PR post		225	3.66	25.74	14.2
GW pre	cont	180	2.55	17.48	14.6
GW post		210	3.00	16.81	17.9

4. Is there a specific pattern of impaired activities of mitochondrial enzymes to account for impaired oxidative metabolism or attenuated increases in oxidative capacity in response to physical training? In order to expand the amount of morphologic as well as oxidative enzyme information from our study, we have modified the original protocol to include performance of muscle biopsies on all subjects recruited for the study. To accomplish this, we have adopted the needle biopsy technique and have been successful in acquiring samples ranging from 70-200 mg. Furthermore, we have adapted our histologic techniques to permit histochemical analyses of such samples and have adopted fluorometric or have miniaturized spectroscopic techniques of enzyme analysis that will enhance the amount of biochemical information available from such samples. To date, we have performed initial needle biopsies on 24 veterans; in an additional 4 veterans we have performed repeat needle biopsy after completion of a 10 week aerobic training protocol. Analysis of these data is incomplete.

Conclusions

We conclude that a factor analysis of symptoms and epidemiological survey of potential toxin exposure will enhance the potential significance of results from this study and provides a highly objective methodology for identifying veterans experiencing symptoms of fatigue, myalgia, and weakness as well as veterans without neuromuscular or other symptoms. Adoption of the needle biopsy technique for acquisition of biochemical and morphologic data and expanding the number of subjects on whom such data will be collected will enhance the capability of detecting and determining the significance of possible differences between symptomatic and asymptomatic veterans.

Evaluation of data to date indicates that Gulf War patients with symptoms of abnormal fatigability have a statistically significant reduction in cycle work capacity and a trend toward a lower peak capacity for oxygen utilization, cardiac output, and a-v O₂ difference which do not reach statistical significance. In contrast, work capacity and fatigue rates in aerobic hand grip exercise in patients and control veterans is similar.

A plausible explanation for a lower cycle exercise capacity in patients would be a lower level of physical conditioning. This interpretation suggests that a program of physical training could be of therapeutic benefit to affected veterans. This hypothesis is supported by the fact that, among subjects who have completed the training protocol, similar improvements in work and aerobic capacity were achieved in both control subjects and patients.

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